TOSOH

TOYOPEARL INSTRUCTION MANUAL

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TOSOH HISTORY

1935	FOUNDING OF TOYO SODA MANUFACTURING CO., LTD.
1936	OPERATION OF NANYO MANUFACTURING COMPLEX BEGINS
1971	SCIENTIFIC INSTRUMENTS DIVISION FORMED, FIRST GPC COLUMN USING TSK-GEL DEVELOPED BY TOSOH
1974	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN PLANT IS COMPLETED
1979	TOSOH DEVELOPS TOYOPEARL MEDIA
1983	TOSOH DEVELOPS HYDROPHOBIC INTERACTION MEDIA
1987	TOSOHAAS US OPERATIONS FORMED IN MONTGOMERYVILLE
1989	TOSOHAAS GMBH OPERATIONS FORMED IN STUTTGART
1995	TOSOH NANYO GEL FACILITY RECEIVES ISO 9001
2000	IN NOVEMBER FORMER TOSOHAAS US OPERATIONS BECOMES TOSOH BIOSEP LLC, A 100% SUBSIDIARY OF TOSOH CORPORATION
2001	IN JANUARY FORMER TOSOHAAS GMBH EUROPEAN OPERATIONS BECOMES TOSOH BIOSEP GMBH, A 100% SUBSIDIARY OF TOSOH CORPORATION
2002/ 2003	TOSOH CORPORATION ANNOUNCES THAT ALL TOSOH AFFILIATED SCIENTIFIC AND DIAGNOSTIC SYSTEM RELATED COMPANIES IN EUROPE, WILL BE UNIFIED UNDER THE NEW NAME TOSOH BIOSCIENCE.
2008	ECOSEC , THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY
2009	20TH ANNIVERSARY OF TOSOH BIOSCIENCE GMBH, STUTTGART

Toyopearl® Instruction Manual

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PACKING

Introduction

Toyopearl chromatographic resins are macroporous polymeric packings for bioprocess chromatography. They are applicable for the laboratory and process scale purifications of globular proteins, peptides, nucleic acids, and other biologically derived materials. These resins are a modified methacrylate polymer which gives the resin a hydrophilic surface due to the presence of ether and hydroxyl groups. It also confers upon the resin excellent pressure/flow characteristics and pH stability.

I. Packing

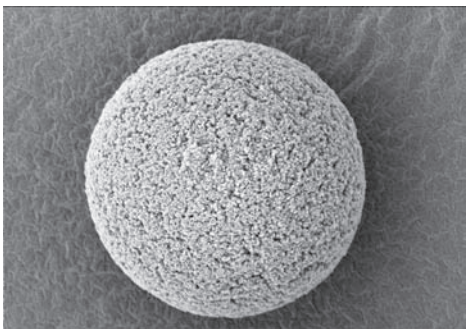
1. Preparation for Packing

1.1 General Considerations for Packing Toyopearl
It is best to pack Toyopearl resins by the application of pressure from 0.5 to 3 bar (7 to 45 psi) across the bed length. Although it is not recommended, Toyopearl resins can be packed by simple gravitational settling.

The equipment components (shown in Fig. 1) required to successfully pack Toyopearl resins are:
a pump, a pressure gauge, a level, glasses, acrylic or PEEK or stainless steel column and a packing reservoir (optional).

FIGURE 1
Equipment required for packing

FIGURE 2
Toyopearl base particle



1.2 Removal of Fines
Tosoh Bioscience recommends that fines be removed. Fines in the gel slurry may obstruct screens or sintered filters and may eventually increase the pressure drop across the column. The following decantation process is required to remove fines from the resin slurry.

a) The settled resin in the shipping containers should be suspended by vigorous agitation or stirring with a rod or paddle (do not use a magnetic stirrer; it will grind the resin, generating fines). Once suspended, transfer the required amount of suspension (approximately 4 volumes suspension = 3 volumes resin) into a container of sufficient volume to hold 4 times the volume of resin being prepared. Add distilled water or buffer to 4 times the resin volume and stir thoroughly.

Example for Fine Removal:
5 liter resin ordered = 7 - 8 liter of suspension in total
(65 - 70 % slurry concentration)
Fill in a 20 liter vessel and fill up with 12 liter water.

b) Allow the resin to settle. Settling time is dependent on the vessel height, the slurry concentration, the solvent, and the resin particle size. The average settling times for Toyopearl resins in water in a typical measurement cylinder are:

Toyopearl	Pore Size	Minutes
Coarse (“C”) Grade	100 µm	15-30
Medium (“M”) Grade	65 µm	30-45
Fine (“F”) Grade	45 µm	45-60
Superfine (“S”) Grade	35 µm	60-90

PACKING

In larger tanks sedimentation of particles takes longer:

	50 % slurry	25% slurry
in water	65 µm particles need 3 - 4 hours per meter SD	65 µm particles need 1,5 - 2,5 hours per meter SD
	35 µm particles need 5 - 7 hours per meter SD	35 um particles need 2 - 3,5 hours per meter
in 1 M NaCl	65 µm particles need 3 - 5 hours per meter SD	65 µm particles need 2 - 3 hours per meter SD
	35 µm particles need 12 - 16 hours per meter SD	35 µm particles need 3 - 7 hours per meter SD
in 1.8 M $(NH_4)_2SO_4$	65 µm particles need 6 - 9 hours per meter SD	65 µm particles need 4 - 8 hours per meter SD
in 20 % ethanol	65 µm particles need 6 hours per meter SD	

SD = Sedimentation Distance

c) Once the resin has settled, carefully decant the supernatant.

FIGURE 3 + 4

Decant

Re-suspend

d) Add three times the resin volume of either distilled water or packing buffer to the decantation vessel, and re-suspend the resin by gentle overhead stirring. Do not use a magnetic stir bar; it will grind the resin, generating fines.

e) Repeat **steps c)** and **d)** at least two more times.

1.3 Buffer Equilibration

When choosing a packing buffer, it is best to choose empirically since the optimal buffer will vary with your specific application. In general, the highest ionic strength mobile phase to be used in the separation (including the cleaning and sanitization steps) is a suitable starting point. Some typical packing buffers are listed in Table 1.

TABLE 1

Typical packing buffer

SEC	
HW-40, HW-50, HW-55, HW-65 and HW-75	0.1 M Na_2SO_4, $NaNO_3$, or NaCl in 50 mM phosphate or Tris buffer
IEC	
DEAE-type, QAE, Q-type, CM-type, SP-type, MegaCap II-SP	1 M NaCl in 50 mM phosphate, Tris, or acetate buffer
HIC	
Ether-650, Phenyl-type, Butyl-type, Hexyl-650, PPG-600,	2 M Na_2SO_4, $(NH_4)_2SO_4$ or NaCl in 50 mM phosphate buffer
AFC	
AF-Tresyl and AF-Epoxy-650	0.5 M NaCl in 0.1 M $NaHCO_3$ or phosphate buffer
AF-Formyl-650, AF-Amino-650, and AF-Carboxy-650, Protein A	1 M NaCl in 100 mM phosphate or $NaHCO_3$ buffer
AF-Chelate-650, AF-Blue HC-650 and AF-Red-650	0.5 M NaCl or 0.2 M glycine in 20 mM phosphate or Tris buffer

1.4 Slurry Preparation

After de-fining the resin, the slurry concentration can be adjusted for packing the column. The slurry concentration is calculated as the volume of settled gel divided by the total volume of the slurry, and the slurry concentration is adjusted as follows:

a) Resuspend the resin slurry in the de-fining vessel and transfer the homogeneous slurry to a graduated cylinder

b) Allow the slurry to settle overnight (>12 hours) for best results.

c) Determine the settled resin volume, and adjust the slurry concentration to 30 - 50 % by adding or removing packing buffer.

d) For packing a column of a given volume, use the following amounts of settled resin:

HW-40, HW-50, HW-55, HW-65, and HW-75F	use approximately 1.1 x the column volume
Ether-650, Phenyl-type, Butyl-type, Hexyl-650, PPG-600, DEAE-type, Q-type, CM-650, SP-type, Giga Cap-type and all affinity	use approximately 1.2 x the column volume
QAE-550C and SP-550C Toyopearl MegaCap II SP 550EC	use approximately 1.25 x the column volume

PACKING

1.5 Alternative Slurry Preparation

a) Re-suspend the resin slurry in the de-fining vessel and transfer the homogeneous slurry to a Büchner funnel or equivalent.

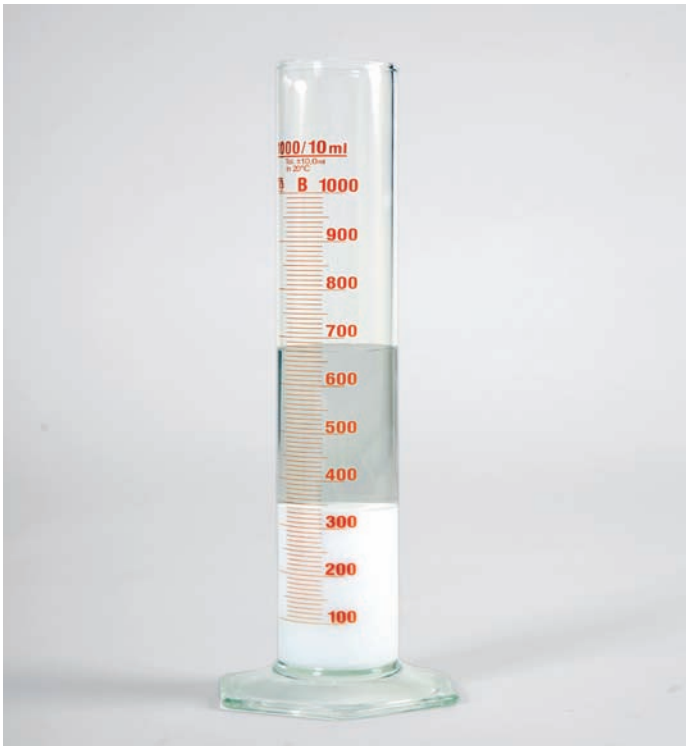
b) Filter the slurry under suction until the slurry becomes a wetcake (all excess liquid has been removed).

c) Weigh out the appropriate amount of resin wetcake (1 g of wetcake ≈ 1 ml of gravity settled gel) using the above table.

d) Transfer the wetcake to a beaker and add enough packing buffer to make a slurry concentration of 30 - 50 %.

FIGURE 5

Settled resin in water



PACKING

2. Packing Procedures

Do not pack Toyopearl like traditional soft gels. For best results Toyopearl should be packed at a higher flow rate and pressure!

TABLE 2

Packing and operating velocities for Toyopearl resins

LABORATORY SCALE

	Resin Type	Column Size (cm ID x cm L)	Grade	Packing velocity flow rate (cm/hr)	Operating velocity (cm/hr)	(ml/min)
SEC	HW-40	2.2 x 60	S (30 µm) F (45 µm) C (75 µm)	30 - 40 60 - 80 120 - 160	10 – 25 25 – 50 50 – 100	0.6 -1.6 1.6 - 3.2 3.2 - 6.4
	HW-50	2.2 x 60	S (30 µm) F (45 µm)	25 - 35 50 - 70	10 – 20 25 – 35	0.6 - 1.3 1.6 - 2.2
	HW-55	2.2 x 60	S (30 µm) F (45 µm)	25 - 35 50 - 70	10 – 20 25 – 35	0.6 - 1.3 1.6 - 2.2
	HW-65	2.2 x 60	S (30 µm) F (45 µm)	20 - 75 40 - 150	10 – 15 15 – 30	0.6 - 1.0 1.0 - 1.9
	HW-75	2.2 x 60	F (45 µm)	40 - 150	15 – 30	1.0 - 1.9
IEC*	DEAE-650, SuperQ-650 CM-650, SP-650 Giga Cap S,CM,Q	2.2 x 20	S (35 µm) M (65 µm) C (100 µm)	400 - 600 800 - 1000 800 -1200	45 - 65 80 - 130 80 - 600	3.0 - 4.0 5.0 - 8.0 5.0 - 40
	SP-550 QAE-550	2.2 x 20	C (100 µm)	700 - 1000	80 - 240	5.0 – 15
	Toyopearl MegaCap II SP-550		EC (100-300 µm)	800 - 1200	80 - 500	5.0 - 30.0
HIC*	Ether-650, Hexyl-650, Butyl-600, Phenyl-650, PPG-600, Butyl-650, SuperButyl-550, PPG-600, Phenyl-600	2.2 x 20	S (35 µm) M (65 µm) C (100 µm)	400 - 600 800 - 1000 800 - 1200 700 - 1000	45 - 65 80 - 130 80 - 500 80 - 240	3.0 - 4.0 5.0 - 8.0 5.0 - 30 5.0 – 15
AFC*	AF-Amino-650, AF-Tresyl-650 AF-Carboxy-650, AF-Blue-650 AF-Formyl-650, AF-Chelate-650 AF-Epoxy-650, AF-Blue-650	2.2 x 10	M (65 µm)	800 - 1000	30 - 130	2.0 - 8.0

** Not all resins are available in all particle sizes.*

PROCESS SCALE

The packing velocity in process scale columns should be at least 1.5 x the operating velocity

Please call our product specialists for your individual discussion.

PACKING

The following descriptions are valid for packing under flow. If you have other equipment, or pack greater than 5 liters, please call our Technical Specialists. We have experience with many different column designs and brands.

TABLE 3

Features of packing methods

Packing Method	Constant Pressure	Constant Velocity	Assisted Gravity
Packing Velocity	fast	fast	slow
Flow Rate Range	up to high	up to high	limited to low
Pump	constant pressure	constant velocity	peristaltic pump
Pressure Gauge	needed	needed	not needed

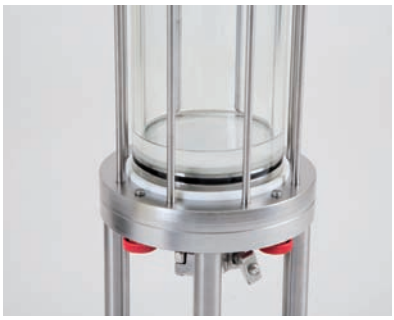
2.1 Constant Velocity / Semi-Constant Pressure Methods

a) If used, place the packing reservoir on the column. The total volume of the column and the reservoir should be sufficient to allow the entire resin slurry to be poured in one operation.

b) Ensure that the column is leveled prior to packing. Wet the bottom frit or screen in the column with buffer. Allow the buffer to drain a few seconds to remove any air bubbles. Plug the outlet of the column and leave 1 - 2 cm of buffer in the bottom of the column.

FIGURE 6

Bubble free, liquid covered bottom frit



c) Resuspend the resin slurry to assure homogeneity.

FIGURE 7

Homogenise slurry

d) Carefully pour the resin slurry slowly down along the inside wall of the column. Prevent air from being trapped in the resin slurry.

e) After the resin slurry is transferred to the column, rinse the inside walls of the column using a squirt bottle containing packing buffer.

FIGURE 8

Pouring the resin

f) Immediately place the flow adapter of the column onto the resin slurry. There should be no trapped air between the flow adapter and the buffer.

g) Open the column outlet, and start the pump. Start slowly to flow packing buffer through the column.

FIGURE 9

Adjusting column

h) Two different Packing Methods can be applied:

Constant Velocity Method

Slowly increase to the final flow rate. This prevents hydraulic shock to the forming bed and prevents uneven packing of the column bed. The flow rate can be ramped up in several incremental changes. These increments will be determined by the size of the column and target flow rate. Some examples are listed in Table 4.

PACKING

Pressure Method

Slowly ramp up to the target pressure. This prevents hydraulic shock to the forming bed, and therefore prevents uneven packing of the column. The pressure can be maintained by manually decreasing the flow rate to keep a constant pressure on the forming bed. The optimal packing pressure for Toyopearl resins is around 3 bar (44 psi) across the bed length.

TABLE 4

Typical packing buffer

Column Size (ID x L)	Media Type	Target Flow Rate (ml/min)	Increment (ml/min)	Hold Time (min)
2.2 cm x 60 cm	HW-55S	2	0.5	0.5
9 cm x 30 cm	QAE-550C	300	50	2
25 cm x 30 cm	DEAE-650M	2,000	400	3

i) After the bed has fully formed, shut off the pump, and close the column outlet.

FIGURE 10

Clear supernatant of sedimenting resin

j) The entire bed should reside in the lower column section if using a packing reservoir. Using a pipette or pump, siphon the supernatant from the upper reservoir. Remove the upper reservoir and the coupling ring.

k) Carefully place the flow adapter into the column, approximately 2 - 3 cm away from the consolidated bed. Avoid introduction of air into the column.

l) Secure the flow adapter in place, begin the pump as described in **Step h** (Pressure Method), and open the column outlet.

FIGURE 11

Flow adapter in place

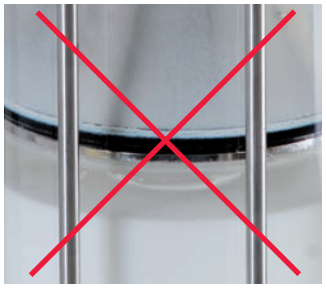
m) The bed will compress further. When compression is complete and pressure is stable, stop the pump and close the column outlet.

n) Carefully loosen the flow adapter seal and lower the adapter near to the resin bed. Take care not to disturb the resin bed when moving the flow adapter.

FIGURE 12 + 13

Bubble free (recommendable)

Air disturbs homogenious settling procedure



o) Repeat **Steps l) - n)**, until there is no further compression of the resin bed from the flow adapter (< 0.5 cm). It will usually take 2-3 iterations until the bed is stable.

p) In the final step lower the adapter 1 - 5 mm into the bed.

q) The column is now ready for efficiency evaluation. (see page 8)

PACKING

2.2 Alternative Packing Method, Assisted Gravity

Due to hardware constraints, it may not be possible to use a reservoir when packing Toyopearl resin. The following method was developed to pack the resin without a packing reservoir.

a) Adjust the resin slurry concentration to 50 %, and gently resuspend the resin with overhead stirring. Do not use a magnetic stirrer!

b) As shown in Figure 14, attach a peristaltic pump to the bottom outlet of the column.

c) Ensure that the column is leveled prior to packing.

d) With the pump running in the upflow direction, backflow packing buffer into the column until it is about 50 % full. Stop the pump.

e) With the pump running at the desired flow rate in the downflow direction, slowly add the homogeneous resin slurry to the column. Pour the slurry down along the inner wall of the column to prevent the formation of air bubbles.

f) When the bed is almost entirely formed, and with approximately 2-3 cm of buffer above the bed, shut off the pump and column outlet valve.

g) Gently rinse down the inside walls of the column with a squirt bottle containing packing buffer.

h) Carefully place the flow adapter into the column, with the adapter just touching the packing buffer.

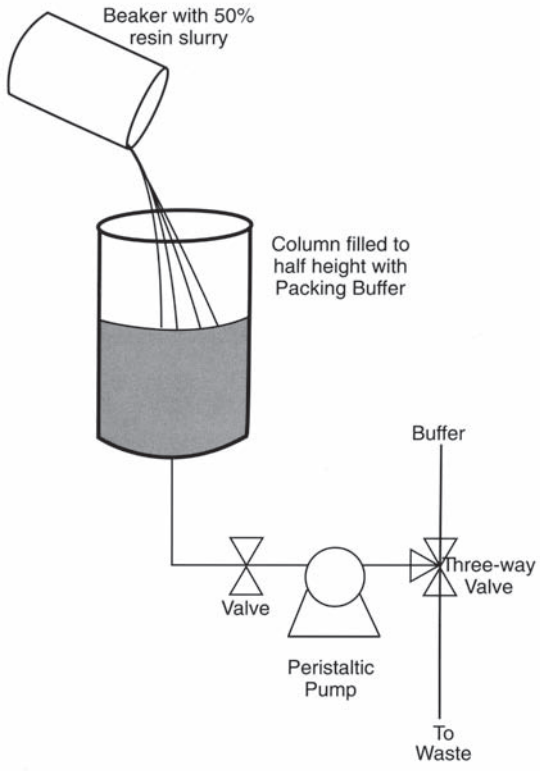
i) With the adapter firmly in place, place the pump in front of the column. Eliminate air in tubing.

j) Start the pump at a low flow rate; open the bottom valve.

k) Slowly ramp up to the target flow rate. This prevents hydraulic shock to the forming bed, and therefore prevents uneven packing of the column. The flow rate can be ramped up in several ml/minute increments over the initial phase of the packing. The size and duration of these increments will be determined by the size of the column which is being packed (see Table 4).

FIGURE 14

Assisted cravity packing method



l) After bed consolidation is complete, stop the pump and shut the bottom outlet.

m)
Loosen the seal on the flow adapter, and gently place the flow adapter onto the resin bed. Be careful not to allow resin past the column seal.

n) Repeat **steps l)** through **m)** until there is no further bed compression from the flow adapter (< 0.5 cm).

o) In the final step lower the adapter 1 - 5 mm into the bed.

p) The column is now ready for an efficiency evaluation. (see page 9)

PACKING

3. Equilibration and Efficiency Evaluation

Once the packing operation is completed, equilibrate the column with 5 - 10 column volumes of low ionic strength buffer. Test the effectiveness of the packing procedure by injecting a sample (0.25 - 1% of the column volume) of a low molecular weight, unretained compound (i.e. acetone, Vitamin B12, sodium chloride), and determine the column plate count and asymmetry as shown in Figure 15. Columns packed according to the above procedures, and operated at linear velocities of 50 – 250 cm/h (depending on the particle size) should have the minimum plate counts listed in Table 5, and asymmetries between 0.8 - 1.5 when tested.

TABLE 5

Typical packing buffer

Mode	Column ID (cm)	S Grade (plates/M)	F Grade (plates/M)	M Grade (plates/M)	C Grade (plates/M)
SEC	2.2	5,000	3,500	-	3,000
	5.5	5,000	3,300	-	-
	10.8	5,000	2,500	-	-
	21.0	4,000	2,200	-	1,500
	31.0	-	2,000	-	1,200
	40.0	-	1,800	-	1,000
IEC	2.2	6,000	-	4,000	2,000
	5.5	6,000	-	4,000	-
	10.8	6,000	-	4,000	-
	21.0	4,000	-	2,600	2,000
	31.0	-	-	2,000	1,000
	40.0	-	-	1,500	750
HIC	2.2	6,000	-	4,000	2,000
	5.5	6,000	-	4,000	-
	10.8	6,000	-	4,000	-
	21.0	4,000	-	2,600	2,000
	31.0	-	-	2,000	1,000
	40.0	-	-	1,500	750
AFC	2.2	-	-	4,000	-
	5.5	-	-	4,000	-
	10.8	-	-	4,000	-
	21.0	-	-	2,600	-
	31.0	-	-	2,000	-
	40.0	-	-	1,500	-

If there is a large deviation from expected plate height number and assymetry factors, please repeat the packing procedure.

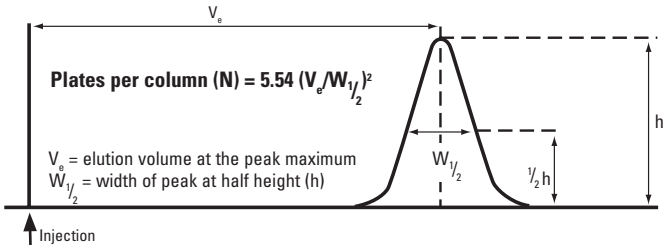
If column diameters > 40 cm are utilized, the number of plates/M can slightly decrease

For further details call our Technical Specialists.

FIGURE 15

How to calculate efficiency & asymmetry Factor

Efficiency



Asymmetry

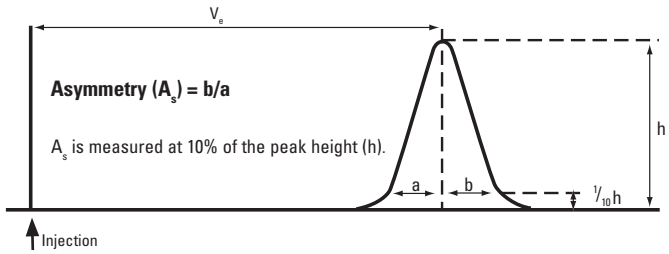


TABLE 6

Troubleshooting performance evaluation

A_s < 0.8	A_s > 1.4
Overpacking the column. Packing at too high pressure. Column bed cracking.	Column not packed "tight" enough. Clogged screens or frits at top or bottom of the column. Small void at top of column. Air pockets in column hardware void spaces. Poor injection technique.
High HETP*	**Low HETP***
Injection sample or detector too far from column. Injection volume too high. Column not packed efficiently.	Probe molecule retained on column due to interaction with functional group or backbone.

*HETP (Height Equivalence of a Theoretical Plate)

COLUMN OPERATION

II. Column Operation

1. Chromatographic Separation

1.1 Size Exclusion Chromatography (SEC)

Equilibrate the resin with 5 - 10 column volumes of an appropriate buffer solution (see Table 1). Size exclusion separations on Toyopearl HW columns are performed under isocratic conditions using buffered salt solutions of moderate ionic strength. Sample volumes are usually 1 - 3% of the column packed bed volume. If retention times are shorter or longer than expected, changes in the mobile phase may be necessary. Please refer to Table 7 for suggested mobile phase changes.

TABLE 7

Non ideal SEC behavior

Observation	Cause/Solution
Retention time is shorter than expected	Sample can be partially or totally excluded from column, confirm MW of sample and use a resin with higher exclusion limit if necessary. Anionic molecules can be repulsed by ionic exclusion, increase the ionic strength of the mobile phase.
Retention time is longer than expected	Cationic molecules can be retarded by ionic attraction, increase the ionic strength of the mobile phase. Hydrophobic molecules can be retarded by hydrophobic attraction, decrease the ionic strength of the mobile phase or add a small percentage (10-20 %) of an organic solvent such as methanol, ethanol, or acetonitrile.

1.2 Ion Exchange Chromatography (IEC)

Equilibrate the column with 5 to 10 column volumes of an appropriate starting buffer solution (Table 8). The elution is performed by increasing the salt concentration or changing the pH of the eluent.

If the ion-exchanger fails to adsorb the desired protein, change the pH of the equilibration buffer to enhance the electrostatic interaction between the protein and the ion-exchanger, or decrease the salt concentration in the equilibration buffer.

TABLE 8

Examples for buffers used in IEC

Resin Type	Buffer	Buffering Range
Cation Exchangers	Acetic acid	4.8 - 5.2
	Citric acid	4.2 - 5.2
	MES	5.5 - 6.7
	Phosphate	6.7 - 7.6
	HEPES	7.6 - 8.2
Anion Exchangers	L-Histidine	5.5 - 6.0
	Imidazole	6.6 - 7.1
	Triethanolamine	7.3 - 7.7
	Tris-HCl	7.5 - 8.0
	Diethanolamine	8.4 - 8.8

1.3 Hydrophobic Interaction Chromatography (HIC)

Equilibrate the column with an appropriate buffer solution containing a concentrated (generally 1 M to 3 M) neutral salt such as one listed in Table 9. High ionic strength enhances the hydrophobic interaction between proteins and the resin and thus facilitates adsorption. Before introducing a sample onto the column, make at least one blank analysis and equilibrate the column in the initial mobile phase.

Elute adsorbed proteins by decreasing the concentration of salt in the eluent. Proteins with lower hydrophobicity are eluted earlier and at higher salt concentrations than more hydrophobic proteins. If the desired protein is not eluted by this method, add a small percentage of organic solvent or nonionic detergent, change the eluent pH, or lower the temperature. See Table 10 for suggestions on what organic solvents, detergents, or chaotropes to use.

If sample profiles are inconsistent, first increase the column equilibration step by using an additional 3 to 10 column volumes of starting eluent. If the desired protein is not adsorbed on the column, increase the concentration of salt in the starting buffer or adjust the pH of the buffer closer to the isoelectric point of the protein.

TABLE 9

Neutral salts used in HIC

Salt *(listed in decreasing order of strength)**	**Comments**
Sodium Citrate	May exhibit high UV absorbency, prone to microbial growth
Ammonium Sulfate	Not stable above pH 8, low UV interference, resists microbial growth, most commonly used salt for HIC
Sodium Sulfate	Solubility is low (1.5 M at 25 °C)
Sodium Chloride	Halide salt can be corrosive to stainless steel, inexpensive
Potassium Chloride	Halide salt can be corrosive to stainless steel

** - based on the Hofmeister series of lyotropic salts*

COLUMN OPERATION

TABLE 10

Mobile phase additives for HIC

Organic Additives	Detergents	Chaotropic Agents
ethanol	Triton X-100	guanidine hydrochloride
methanol	octyl glucoside	tetraethylammonium chloride
isopropanol	Tween 20	urea
n-butanol	SDS	potassium thiocyanate
acetonitrile	CHAPS	
ethylene glycol	Emulgen 911	
	CTAB	
	Lubrol PX	

1.4 Affinity Chromatography (AFC)

Included among the Toyopearl affinity resins are both group specific ligand resins (Chelate, Red and Blue-HC), and resins with surface chemistries that allow attachment of custom ligands by the end user.

Contact Tosoh Bioscience Technical Service for information concerning coupling chemistries for the attachment of ligands to Formyl, Carboxy, Amino, Epoxy and Tresyl Toyopearl.

Equilibration

AF Red, AF Blue-HC and Chelate resins should be equilibrated with 3 - 5 column volumes of the appropriate starting buffer, such as phosphate or Tris, with little or no salt.

The dye affinity chromatographic resins may release a small amount of conjugated dye during storage. Be sure to wash the dye affinity columns before each use to remove the released dye. Wash a column containing new resin with 1 M sodium chloride or 1 M potassium chloride. Use 2 M potassium chloride or 4 M urea for washing used resin. Equilibrate a column containing old or new resin with an appropriate starting buffer, such as 20 mM phosphate at pH 7.5.

Loading and Elution

After applying the sample, wash the column with 3 - 5 column volumes of starting buffer to remove unadsorbed impurities. Two kinds of elution methods are commonly used in affinity chromatography: nonspecific and specific.

Nonspecific elution generally is achieved by increasing the salt concentration in the eluent. Most proteins are eluted with a solution containing 2 M sodium chloride or 3 M potassium chloride. Proteins not eluted with these eluents can be eluted with solutions listed in Table 11.

In specific elution, an enzyme is eluted with a solution containing its substrate or coenzyme. A substrate or coenzyme concentration below 10 mM usually is sufficient for elution.

TABLE 11

Eluents for exhaustive elution from AF Toyopearl resins

Choice:	2 M KCl or 3 M NaCl
Choice:	1 % Triton X-100 / 1 M NaSCN / 75% ethylene glycol / 4 M urea or 0.1 M NaOH / 4.2 M $(NH_4)_2$ SO_4

2. Cleaning

Toyopearl resins can be cleaned in the column or removed from the column and treated in bulk. The cleaning method and duration of treatment depend on the extent of contamination. At least three bed volumes of cleaning solution are typically employed in column washing procedures.

SEC Resins

In most cases, the resins can be cleaned simply by washing with distilled water to desorb remaining proteins. For more tenaciously bound materials, the following solutions may be required:

Ionically-bound materials

For moderately bound materials, 0.5 - 1 M aqueous salt solutions can be used to clean the resin. For more strongly bound materials, 0.1 - 0.5 M sodium hydroxide or 0.1 - 0.5 M hydrochloric or sulfuric acid is appropriate. Under no circumstances should nitric acid be used to clean Toyopearl resins! Nitric acid can react violently with Toyopearl resins. Because acids sometimes cause protein aggregation, first use an alkaline solution for removing proteins.

Hydrophobically-bound materials

About 10 - 20 % of an alcohol such as ethanol, methanol, or isopropanol can be used to remove hydrophobic materials. Solvents such as acetonitrile and acetone can also be used. It is important to remember that solvents can sometimes cause protein aggregation.

After using any base, acid, or organic solvent, use distilled water as a final rinse.

IEC Resins

For moderate contamination, wash with 0.5 - 1 M sodium chloride, then equilibrate with the starting buffer. For severe contamination, wash with 0.1 - 0.5 M sodium hydroxide, then with 0.1 - 0.5 M sodium chloride, then equilibrate with the starting buffer.

For extremely severe contamination of DEAE and QAE resins, wash with 0.1 - 0.5 M sodium hydroxide, then distilled water, then 0.1 - 0.5 M hydrochloric acid, and then 0.1 - 0.5 M sodium chloride. Equilibrate with the starting buffer.

A high salt mobile phase can be used as a final rinse to assure the correct counter ion is present.

COLUMN OPERATION

HIC Resins
In most cases, the resins can be cleaned simply by washing with distilled water to desorb remaining proteins. For more tenaciously bound materials, the following solutions may be required:

Ionically-bound materials
For moderately bound materials, 0.5 - 1 M aqueous salt solutions can be used to clean the resin. For more strongly bound materials, 0.1 - 0.5 M sodium hydroxide or an appropriate acid such as hydrochloric or sulfuric is appropriate. Under no circumstances should nitric acid be used to clean Toyopearl resins! Because acids sometimes cause protein aggregation, first use an alkaline solution for removing proteins.

Hydrophobically-bound materials
10 - 40% of an alcohol such as ethanol, methanol, or isopropanol can be used to remove hydrophobic materials. Solvents such as acetonitrile and acetone can also be used. It is important to remember that solvents can sometimes cause protein aggregation. Non-ionic detergents may also be used for cleaning.

After using any base, acid, or organic solvent, use distilled water as a final rinse.

AFC Resins
High concentrations of neutral salts, chaotropes, or detergents such as those listed in Table 9 should be used as eluents prior to extensive cleaning efforts. Remaining protein contaminants adsorbed on the resin can be removed by washing with two column volumes of 0.5 M sodium hydroxide followed by distilled water. Sodium hydroxide should be used with AF-Heparin and AF-Protein only in cases of extreme contamination.

3. Storage

SEC, IEC, and HIC
Store the column or used bulk resin in distilled water containing a bacteriostatic agent, such as 20% ethanol, preferably at 4°C to 25°C.

AFC
Store the column or used bulk resin in a neutral solution of 1 M sodium chloride or potassium chloride containing a bacteriostatic agent, such as 20% ethanol, preferably at 4°C to 10°C.

For AF-Formyl 650M, store the column or used bulk resin in a neutral solution of 1 M sodium chloride or potassium chloride in 1% gluteraldehyde, preferably at 4°C to 10°C.

Please note that dye affinity chromatographic resins may release a small amount of dye during storage. Be sure to wash the dye affinity resin before each use to remove any released dye.

COLUMN OPERATION

4. Sterilization / Depyrogenation / Preservative Removal / Column Frits

Sterilization
Toyopearl resins can be sterilized by autoclaving at 121°C for 20 min. without altering their properties. Alternatively, columns already packed may be exposed to 200 ppm sodium hypochlorite for periods up to 12 hours without loss of function.

Depyrogenation
Toyopearl resins are recommended for use from pH 2 to 12. However, short exposures (< 12 hours) to higher pH (0.5 N NaOH) are acceptable for depyrogenation. Typically endotoxin levels are reduced by at least 4 logs following a 4-hour treatment with 0.5 N NaOH followed by a wash with 3 column volumes of endotoxin-free equilibration buffer.

Preservative Removal
Shipping solvents for Toyopearl resins contain 20% ethanol (with exception of some affinity products). The resin preparation procedures outlined in this document will reduce the ethanol level in the packed column effluent.

Column Frits
Pressure-related problems are often caused by clogged column frits. Remove the frits and clean thoroughly as recommended by the column manufacturer. If the problem persists, replace the frits.

TOYOPEARL PRODUCT OVERVIEW

IEC

Toyopearl Resin	Pore Size
Anion-Exchangers	
SuperQ-650 (S, M, C),	400 Å
QAE-550C,	500 Å
DEAE-650 (S, M, C),	1.000 Å
GigaCap Q-650M (M=75 µm)	1.000 Å
Q-600C AR	750 Å

S = 35 µm, **M** = 65 µm, **C** = 100 µm

Cation-Exchangers	
CM-650 (S, M, C),	1.000 Å
SP-650 (S, M, C),	1.000 Å
SP-550C,	500 Å
MegaCap II SP-550EC,	500 Å
GigaCap S-650M, (50-100 µm)	1.000 Å
GigaCap CM-650M, (50-100 µm)	1.000 Å

S = 35 µm, **M** = 65 µm, **C** = 100 µm, **EC**= 200 µm

AFC

Toyopearl Resin	Pore Size
Toyopearl Reactive Resins	
AF-Amino-650M,	1.000 Å
AF-Carboxy-650M,	1.000 Å
AF-Formyl-650M,	1.000 Å
Toyopearl Activated Resins	
AF-Epoxy-650M,	1.000 Å
AF-Tresyl-650M,	1.000 Å
Toyopearl Ready to use Resins	
AF-BlueHC-650M,	1.000 Å
AF-Chelate-650M,	1.000 Å
AF-HeparinHC-650M,	1.000 Å
AF-Red-650ML,	1.000 Å

M= 65 µm, **ML** =65 µm

HIC

Toyopearl Resin	Pore Size
Ether-650 (S, M),	1.000 Å
PPG-600M ,	750 Å
Phenyl-600M,	750 Å
Phenyl-650 (S, M, C),	1.000 Å
Butyl-650 (S, M, C),	1.000 Å
Butyl-600M,	750 Å
SuperButyl-550C,	500 Å
Hexyl-650C,	1.000 Å

S = 35 µm, **M** = 65 µm, **C** = 100 µm

SEC

Toyopearl Resin	Pore Size
HW-40 (S, F, C),	50 Å
HW-50 (S, F),	125 Å
HW-55 (S, F),	500 Å
HW-65 (S, F, C),	1.000 Å
HW-75 (F),	> 1.000 Å

S = 30 µm, **F** = 45 µm, **C** = 75 µm

PROCESS COLUMN INSTALLATIONS

Column Manufacturer	Column Type	various bed dimensions (ID x L in cm)	Column Performances Af	plate count [N/m]	Resin Type
BioRad					
	InPlace/Geltec	20 - 45 x 15 - 25	0,8-1,4	3.000-4.000 (60 cm/h-salt)	HIC - 65 µm
		130 x 24	1,1-1,2	3.500-3.900 (300 cm/h-salt)	IEC - 65 µm
GE Healthcare Lifesciences					
	AxiChrom	60 x 20	1,1	8.000 (100 cm/h-salt)	HIC - 65 µm
	BPG	20 - 30 x 11 - 25	0,9-1,3	4.000-11.000 (40cm/h-salt)	IEC/HIC - 65 µm
	Chromaflow	40 - 80 x 15 - 24	1,1-1,4	3.000-5.000 (100cm/h-salt)	IEC - 65 µm
	Index	20 - 35 x 28 - 32	1,3-1,4	14.-20.000 (20cm/h-acetone)	IEC - 20 µm
		20 x 15 - 25	0,8-1,6	3.000-6.000 (100cm/h-acetone)	IEC/HIC - 35/65 µm
Merck					
	Superformance	20 - 30 x 15 - 30	1,0-1,3	2.500-3.500 (100cm/h-acetone)	IEC - 65 µm
		20 x 30	1,2	7.000 (250cm/h-acetone)	IEC - 20 µm
Millipore					
	IsoPak / Access	44 x 25	1,2-1,5	6.000-9.000 (acetone-60 cm/h)	IEC - 35 µm
		44 x 13 - 30	1,1-1,4	3.000-8.000 (130-20 cm/h)	IEC/HIC - 65 µm
		100 - 160 x 15 - 25	1,2-1,4	4.000-6.000 (salt-60 cm/h)	IEC/HIC - 65 µm
		140 x 25	1,4-1,7	5.000-7.000 (salt-60cm/h)	IEC - 35 µm
		160 x 13 - 15	1,0	600-900 (acetone-100 cm/h)	IEC - 100 µm
		200 x 30	1,2-1,4	4.000-5.500 (100cm/h-salt)	HIC - 65 µm
	QuikScale	20 - 30 x 13 - 20	1,2-1,6	4.000-10.000 (acetone-100 cm/h)	HIC - 35 µm
		14 - 30 x 13 - 33	1,3-1,6	2.500-5.000 (acetone-100 cm/h)	IEC - 65 µm
		63 x 17	1,2-1,4	2.500-4.000 (acetone-130 cm/h)	IEC - 65 µm
	Moduline	140 x 20 - 25	0,8	5.000-6.000 (salt-30cm/h)	IEC - 65 µm
Pall/Euroflow					
	Resolute™	40 - 80 x 12 - 32	1,1	16.000-19.000 (salt-60cm/h)	HIC - 35 µm
		40 - 80 x 14 - 32	0,8-1,2	3.000-7.000 (salt-30cm/h)	HIC/IEC - 65 µm
		40 - 100 x 21 - 28	1,0-1,2	1.000-3.000 (salt-100cm/h)	IEC - 100/200 µm
		100 - 140 x 20 - 25	1,0-1,3	3.000-7.000 (salt-80cm/h)	HIC - 65 µm
Peak Biotech/ DAN Process					
	LPLC-DAC	30 x 19 - 21	1,3-1,4	13.000-17.000 (salt-100cm/h)	HIC/IEC - 20 µm
		30 x 20	1,2-1,8	6.000-8.000 (salt-100cm/h)	HIC/IEC - 35 µm
		30 x 20	1,2	4.000 (salt-80cm/h)	IEC - 65 µm
Proxcys					
	CRIO - radial flow	5 - 20 liter BV, 6 - 11,6 cm L	1,0-1,2	3.000-7.000 (salt-100cm/h)	IEC - 65 µm

These examples show real values for any packing condition given. It need not to be the achievable optimum.

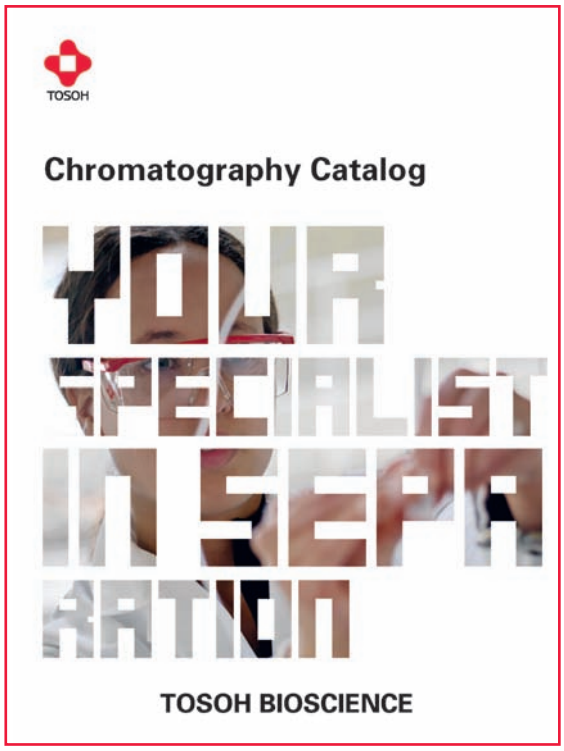
We have more than 10 years of experience in packing production columns of various manufacturers.
Please call our specialist for your individual discussion. In addition we assist you on-site.

YOUR NOTES

To get an overview about the whole range of our:

bulk media for biopurification, please request our **process catalog**

columns and small bulk media, please request our **chromatography catalog**



For a deeper insight into applications and all questions related to the practical use of TSK-GEL and Toyopearl check our website **www.tosohbioscience.com** and the related **catalogues** or **instruction manuals**.

Our **technical experts** are happy to discuss your specific separation needs via

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